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Changing Mechanisms of Action as a Strategy for Sequential Targeted Therapy of Metastatic Renal-Cell Carcinoma

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Additional information is available at the end of the chapter

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1. Introduction

Renal cell carcinoma (RCC) affects more than 200.000 people annually worldwide resulting in 102.000 deaths each year. Men are twice as frequently affected as women; population aged between 50 and 70 years is most frequently affected. Obesity, hypertension, tobacco smoking and certain occupational exposures have been shown to increase one's risk for developing RCC. Rarely RCC develops as a part of the familiar syndrome (e.g. von Hippel-Lindau) [1,2].

Treatment of renal cell carcinoma has changed dramatically over the past few years. Until 2005 cytokine therapy (interferon (IFN-α) or interleukin (IL-2)) was the only (IFN-α) or interleukin (IL-2) was the only available treatment for mRCC patients. Treatment with cytokines was associated with little clinical benefit together with substantial side effects; even treatment related deaths were not infrequent. Treatment options for second line therapy were very limited; patients could be treated only with another cytokine or best supportive care. Responses to second line cytokine therapy were modest. Fewer than 4% of patients had partial response and < 12% had stable disease [2,3].

Lack of effective therapy together with better knowledge about the cancer biology led to the development of new targeted agents. Since the start of the "targeted era" development of new therapies evolved swiftly. Better treatment results in the first line therapy are allied to the better outcome of the patients on subsequent lines of treatment. Prognosis of patients improved and mRCC is becoming more a chronic type of disease, rather than a rapidly progressing and fatal one [3].

Despite rapid progress in development of new treatments, many questions still remain unanswered. Patients on targeted therapies progress some time during their treatment and

mRCC is considered an incurable disease [4]. In trying to overcome this, the mechanism of action and especially mechanisms of resistance to targeted therapies, need to be studied and explained even more in detail [3-7].

In this chapter evidence on sequential therapy after progression to the first line will be presented with the emphasis on changing mechanism of action. Additionally, mechanisms of resistance to targeted therapies and therapeutic options to overcome resistance will be discussed.

2. Molecular biology of renal cell carcinoma

Most of the knowledge about molecular biology comes from the studies of a hereditary form of renal cell carcinoma. Studies of families with inherited RCC over the past twenty years lead to the identification of five inherited renal cancer syndromes and their related genes. Description of all five syndromes is beyond the scope of this chapter; only Von Hippel-Lindau syndrome will be explored [2].

2.1. Von Hippel-Lindau tumor suppressor gene

The von Hippel-Lindau (VHL) disease is a rare, autosomal dominantly inherited disease. Individuals with this syndrome are predisposed to development of multiple benign and malignant tumors. Most common are clear cell renal tumors, retinal and central nervous system hemangioblastomas, pheocromocytomas, pancreatic neuroendocrine tumors, endolymphatic sac tumors and pancreatic and kidney cists. VHL occurs in 1 in 36.000 and symptomatic disease develops in 70% of affected persons by the age of 60 years. Bilateral RCC develop in 25-45% of VHL patients. VHL results from mutation in the von Hippel-Lindau gene on chromosome 3p25-26. The VHL gene discovered in 1993 is a tumor suppressor gene; both copies of gene must be inactivated for tumor initiation. Different germline mutations predisposing to VHL include; large deletions, protein-truncating mutations and missense mutations that exchange the amino acids in the VHL protein. More than 1000 different mutations have been identified until now. According to the type of mutation, patients are classified in different groups, predisposed to different types of tumors. Group of patients bearing deletions or nonsense mutations, most often develop RCC [2,8].

The research on VHL gave light to the inside of molecular biology of sporadic kidney cancer. It is known, that loss of VHL function, including somatic mutations and epigenetic defects, is found in 70–90% of the sporadic clear cell RCC [8]. The pathophysiologic mechanism of such strong association is currently not very understood [8,9].

The VHL protein pVHL has several functions. The most studied is its role in the regulation of hypoxia inducible factor (HIF1α), member of transcription factors family. At normal cellular oxygen levels, pVHL binds to HIF1α and causes its degradation. In low oxygen or in the case when VHL gene is mutated pVHL does not bind to HIF1α. Consequently HIF1α dimerise with HIF1β and activate the transcription of genes involved in vessel development (vascular endothelial growth factor, platelet-derived growth factor B, erythropoietin) and genes

involved in glucose uptake and metabolism. Up-regulation of targeted genes involved in neo-vascularization by HIF1α offers the explanation of high vascularity of RCC [2,8]. Beside this, pVHL has numerous other functions in the processes of regulation of extracellular matrix, senescence, phosphorylation enhancers and other. The importance of many physiologically relevant functions of pVHL is at present difficult to interpret [8].

Besides VHL, six other genes have been found to predispose to RCC (MET, FLCN, FH, SDH, TSC 1 and TSC 2). These genes interact trough common nutrient and energy sensing pathways. Understanding of the molecular mechanisms by which these genes interact in these pathways has enabled the development of targeted therapies [2].

2.2. VEGF-R pathway

Loss of both alleles of VHL gene leads to up-regulated transcription of growth factors such as VEGF, PDGF and TGF-α. These factors bind to their tyrosine kinase receptors. This leads to downstream signalling and ultimately to effects such as increased angiogenesis, increased cell proliferation and decreased apoptosis. As described previously pVHL mutations are inevitably connected to flawed HIF inactivation which results in production of VEGF. VEGF is the most prominent angiogenesis regulator. Its function is mediated through two tyrosine kinase receptors VEGF-R1 and VEGF-R2 in vascular endothelial cells. VEGF in the beginning binds to VEGF-R2, which promotes endothelial cell proliferation, migration and vascular permeability. In the next step VEGF binds to VEGF-R1 to assist the organization of new capillaries [9].

2.3. mTOR pathway

mTOR is another regulator of HIF 1α, its signalling activity increases the cellular levels of HIF 1α, which worsens the already high levels of it because of absence of pVHL function. mTOR is a serine/threonine kinase that has a key function in apoptosis, cell growth and tumor proliferation. mTOR forms complexes with regulatory associated proteins named mTORC1 and mTORC2. mTORC 1 can be activated by growth factors including VEGFR, PDGFR, EGFR and IGFR and nutrients trough phosphatidylinositol-3 kinase/Akt (PI3K/Akt) pathway. Activated mTORC1 stimulate protein synthesis, entrance into G 1 phase, and proteins that regulate apoptosis [9].

3. Development of systemic therapy in mRCC

3.1. Chemotherapy

The successes on other solid tumors led researches to the assumption chemotherapy would be effective also in mRCC. Chemotherapeutic trials were conducted between 1983 and 1993. Different agents; bleomycin, cisplatin, 5-FU, gemcitabine and vinblastine have been tested. Results were disappointing; less than 10% of patients had clinical benefit in all of these trials. Response rates in the range of 10 to 15% have been achieved with combination of two agents.

Today chemotherapy has no role in the treatment of mRCC patients and is not part of the everyday clinical practice [2].

Several mechanisms have been discovered to be responsible to the resistance of RCC cells to chemotherapy. Beside increased detoxification, altered targets and impaired apoptosis pathways, increased expression of transporting proteins play an important role. P-glycoprotein is a 170-kD membrane glycoprotein that acts as a pump that expels chemicals like vinblastine out of the cell [2].

3.2. Cytokines

The interest in interferon in the treatment of RCC came when sporadic responses in patients with RCC on leucocyte interferon, were observed. Natural interferon produced from donor's leucocytes, was later substituted with recombinant. Different forms and dosages were tested and no major differences between them were observed. Uniformly response rates ranged from 0 to 29% with few complete and very few durable responses. Some trials suggested that certain group of patients have larger benefit (good performance status, prior nephrectomy and restricted metastases to the lungs), but this was not a uniform finding. Today interferon as mono-therapy is not widely used, because of the low efficacy coupled with high toxicity [2].

IL-2 was discovered in 1979 and it soon became clear that it could be effective in the treatment of RCC. Response rates of 33% have been reported in the initial trials. Later multicentric trials reported response rates in 7-19% of patients. In small number of patients responses were complete and durable; 7-9% of all patients did not relapse even after 10 years and these patients are considered to be cured from cancer. Unfortunately until today the selection of patients likely to have durable responses is not possible, because patient and tumor characteristics that predict best responses to IL-2 have not been identified yet [2]. Beside uncertain responses, unfavourable toxicity profile limits the use of IL-2. Patients treated with high doses of IL-2 may experience vascular leak syndrome, hypotension, multiorgan dysfunction and a variety of other toxicities. In the two decades, when IL-2 was the standard therapy of mRCC patients were selected on safety bases (performance status, co-morbidities), tumor histology (clear cell), risk scores (e.g. Memorial Sloan Kettering Cancer Center) and patient preferences [2,10].

3.3. VEGF targeted therapy

It is not surprising that several agents targeting VEGF demonstrate activity in RCC. As described in previous sub chapter there is direct link between VHL mutation and up regulation of angiogenesis- promoting proteins including VEGF and PDGF. VEGF is the main factor responsible for tumor angiogenesis and PDGF is signalling protein for perycites, structural supporting cells for blood vessels. VHL is mutated in most of the patients with RCC [2,10,11].

3.3.1. Sunitinib

Sunitinib is a potent multi-kinase inhibitor including platelet-derived growth factor receptor (PDGFR) α and β, stem cell factor receptor (c-KIT), FMS-like tyrosine kinase-3 (FLT-3), VEGF receptors 1,2,3, colony stimulating factor (CSF-1R) and neurotropic factor receptor. Large multicentric phase 3 trial in which 750 patients were randomized in a 1:1 fashion between treatment with IFN-α and sunitinib, demonstrated its superiority over IFN-α. Overall response rate was 31% in the sunitinib and 6% in the IFN group (p<0.0001).The median PFS in the sunitinib group was 11 and in IFN-α 5 months. Difference was observed also in overall survival (median 26.8 months in sunitinib and 21.8 months in the IFN group, p=0.051). The most common adverse events were fatigue, diarrhea, mucositis, hand-foot syndrome and hypertension [12,13]. Sunitinib was approved by FDA in 2007 and is today standard of care in the first-line treatment of mRCC [2,9].

3.3.2. Pazopanib

Pazopanib is an oral multitargeted tyrosine kinase inhibitor that targets VEGFR 1,2,3, PDGFR α and β and c-KIT. Approval of pazopanib in 2009 was based on a phase III trial in which 435 patients were randomized (2:1) to receive either pazopanib 800 mg once daily or placebo. The median PFS of 9.2 months in the pazopanib group was significantly longer than in placebo group where PFS was 4.2 months (p<0.0001). Main side effects were diarrhoea in half, hypertension in 40% and nausea, anorexia, vomiting and fatigue in 20% of patients [14]. Grade 3 hepatotoxicity was also reported. Pazopanib is recommended in the first line treatment of mRCC [2,14].

3.3.3. Bevacisumab

Bevacisumab is a recombinant monoclonal antibody that binds circulating VEGF protein and neutralizes it [15]. In the AVOREN trial 649 previously untreated patients were randomized to receive either bevacisumab every two weeks and IFN-α or placebo and IFN-α. Differences in PFS (10.2 vs. 5.4 months) and ORR (31 vs. 13%) were significantly better in bevacisumab group (p<0.0001 for both parameters) [16]. The second trial conducted by Cancer and Leukemia Group B was similar. PFS of 8.5 months in the bevacisumab was statistically significant better than in the IFN monotherapy group (PFS 5.2 months, p<0,0001). Differences were also demonstrated comparing ORR favoring bevacisumab group (25.5 vs. 13.1% p<0,0001). Fatigue, anorexia, hypertension and proteinuria were among the most common side effects and more prominent in the combination group [2,11,16,17].

3.3.4. Sorafenib

Sorafenib is a small molecule, oral multikinase inhibitor that inhibits VEGFR 1,2,3, PDGFR-β, RAF, serine/threonine intracellular kinase, FLT-3, cKIT and RET. Sorefenib was tested in a phase III trial (TARGET), 903 patients with mRCC resistant to standard therapy were randomized to receive sorafenib twice daily or placebo. PFS in the sorafenib group was 5.5 months and in placebo group 2.8 months (p<0.000001). Difference in OS did not reach statistical

significance. Major side effects of sorafenib were rash, hand-foot syndrome, fatigue, diarrhoea and hypertension [18].

3.3.5. Axitinib

Axitinib is a second line inhibitor of VEGFR 1 and 2 and is approved in the second line treatment of mRCC. Axitinib was compared to sorafenib in a phase III trial (AXIX). 723 patients previously treated with suntinib, bevacisumab plus IFN, temsirolimus or cytokines that progressed, were randomized to receive axitinib or sorafenib 400 mg. Median PFS in the axitinib group was 6.7 months and was statistically significant better than in sorafenib group (4.7 months, p<0.0001). Patients in axitinib group had more hypertension, diarrhoea, dysphonia, fatigue and nausea, while patients in sorafenib had more hand foot syndrome, rash and alopecia [19].

3.4. mTOR targeted therapy

Abnormal functioning of signaling pathways contributes to many malignancies including RCC [20-22]. The mammalian target of rapamycin (mTOR) is a protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription [2]. The disruption of mTOR signaling leads to suppression of the production of proteins that regulate progression of the cell trough the cell cycle and angiogenesis [22].

3.4.1. Temsirolimus

Temsirolimus, an mTOR inhibitor was approved for the treatment of mRCC in the 2007. Global Advance Renal Cell Carcinoma (ARCC) was a phase III trial of temsirolimus in previously untreated mRCC. Patients were randomized to receive either IFN-α, temsirolimus or both. PFS in the groups receiving temsirolimus was significantly longer than in the IFN group (3.7 months in temsirolimus groups vs. 1.9 months in the IFN group; p=0.0019). Patients treated with temsirolimus alone had better overall survival than patients treated with IFN alone (10.9 months vs. 7.3 months, p=0.096). Toxicity was greater in the combination group and included rash, stomatitis, pain, infection, peripheral edema, thrombocytopenia, hyperlipidemia, hypercholesterolemia and hyperglycemia [20].

3.4.2. Everolimus

Everolimus is an oral mTOR inhibitor approved for the treatment of mRCC in the second line after progression on sunitinib or sorafenib. Everolimus treatment was tested in phase III trial named RECORD-1. 410 patients which had progressed to previous treatment were randomly assigned to receive either everolimus or placebo. Median PFS in the everolimus group was 4.9 months, in placebo group 1.9 months (p<0.001). The median OS was not significantly different between the two groups (14.8 in everolimus and 14.4 in placebo group, p=0.126) The most common side effects of everolimus were stomatitis, rash, fatigue, asthenia and diarrhea [21,22].

Summary of phase III trials is presented in Tables 1 and 2.

	Author	Name	Treatment Arm	Prior Therapy
Sunitninib	Motzer et.al. (2007)		Sunitinib IFN-α	None
Pazopanib	Sternberg et.al. (2010)		Pazopanib Placebo	None or Cytokine
Bevacisumab	Escudier et.al. (2007)	AVOREN	Bevacisumab plus IFN-α Placebo plus IFN-α	None
Sorafenib	Escuder et.al. (2006)	TARGET	Sorafenib Placebo	Cytokine
Axitinib	Rini et.al. (2011)	AXIS	Axitinib Sorafenib	Sunitinib or Bevacisumab+IFN or temsirolimus or cytokines
Everolimus	Motzer et.al. (2008)	RECORD-1	Everolimus Placebo	Sunitinib or Sorafenib
Temsirolimus	Hudes et.al. (2007)	ARCC	Temsirolimus IFN-α Temsirolimus plus IFN-α	None

Table 1. Phase III trials of targeted agents in mRCC

4. Mechanism of resistance to targeted therapies

Large advances in treatment results achieved with targeted therapies in mRCC are remarkable, but still between a third and two-thirds of patients with mRCC have tumors refractory to anti-VEGF and mTOR inhibitors from the beginning of treatment and all patients develop drug resistance and relapse some time during the course of their disease. Research of the mechanisms of resistance is very important in planning the development of new targeted agents [3, 23,24]. Most of information about drug resistance in mRCC known today is from the preclinical studies or studies on patients with different types of cancer, where targeted therapies are being in clinical practice for longer time (e.g. breast cancer). This is partially due to the rapid approval of targeted agents in mRCC which surpassed understanding of the mechanisms of response and resistance [3].

Until now two types of resistance to targeted therapy have been determined, so called intrinsic and extrinsic resistance [3].

	Objective Response Rate	Progression Free Survival	Overall Survival	Most Common Adverse Events of Experimental Drug
Sunitninib	31% Sunitinib 6% IFN	11 months Sunitinib 5 months IFN P<0.0001	26.4 months Sunitinib 21.8 months IFN P=0.051	Diarrhea, fatique, nausea, stomatitis, vomiting
Pazopanib	30% Pazopanib 3% Placebo	9.2 months Pazopanib 4.2 months Placebo P<0.0001	21.1 months Pazopanib 18.7 months Placebo	Diarrhea, hypertension, hair color change, nausea, anorexia
Bevacisumab	31% Bev+IFN 13% Placebo+IFN	10.2 months Bev+IFN 5.4 months Placebo+IFN P<0,0001	23.3 months Bev+IFN 21.3 months Placebo +IFN P=0.13	Fatique, pyrexia, anorexia, bleeding, asthenia
Sorafenib	10% Sorafenib 2% Placebo	5.5 months Sorafenib 2.8 months Placebo P<0,01	17.8 months Sorafenib 15.2 months Placebo P=0.15	Diarrhea, rash, fatigue, hand-foot syndrome
Axitinib	19% Axitinib 9% Sorafenib	6.7 months Axitinib 4.7 months Sorafenib P<0.001	Not reported	Diarrhea, hypertension, fatigue, decreased apetite, nausea
Everolimus	1% Everolimus 0% Placebo	4.9 months Everolimus 1.9 months Placebo P<0,0001	14.8 months Everolimus 14.4 months Placebo P=0.177	Stomatitis, rash, fatigue, asthenia, diarrhea
Temsirolimus	8.6% Tem 4.8% IFN 8.1% Tem+IFN	3.7 months Tem 1.9 months IFN 3.7 months Tem+IFN P=0.0001 (between Tem and IFN)	10.9 months Tem 7.3 months IFN 8.4 months Tem+IFN P=0.08 (between Tem and IFN)	Asthenia, rash, anemia, nausea, anorexia

Table 2. Phase III trials of targeted agents in mRCC

4.1. Intrinsic resistance

Intrinsic resistance (primary resistance) occurs when tumor does not respond to the targeted therapy from the beginning of the treatment. Lack of the clinical benefit, even a short-lasting one is observed in these patients. Roughly 25% of patients are resistant to therapy; no response is detected on first evaluation after 2-3 months [23]. This type of resistance has not been explained entirely jet.

In the case of the resistance to VEGF inhibitors and TKI-s pre-existing pro-angiogenic factors, such as fibroblast growth factor-2 promote tumor angiogenesis. Pre-existence of pro-angiogenic factors compensate for the inhibition of VEGF signaling and thus allow angiogenesis to continue [3,23]. Pre-existing inflammatory cells may also contribute to the angiogenesis by expressing pro-angiogenic factors. In pre-clinical trials mRCC tumors that were not responsive

to anti-VEGF antibody were associated with increase in infiltrating CD11b + GR1 + myeloid cells, which expressed several pro-angiogenic factors [23].

The proposed mechanisms of resistance to inhibitors of mammalian target of rapamycin (mTOR) include the presence of redundant signaling pathways, presence of KRAS or BRAF mutations, loss of phosphatase and tension homologue deleted on chromosome ten (PTEN), low cellular levels of p27 or 4E-bp1 and overexpression of eIF4E [3].

Intrinsic resistance to anti-angiogenic factors and mTOR inhibitors is widespread and leads to poor patient outcome. Alternative pathways should be considered in this patients such as targeting RAF and MEK or PI3K/AKT. Including patients with resistant tumors in clinical trials testing these new agents that target these pathways is strongly recommended [23].

4.2. Extrinsic resistance

All patients who initially have clinical benefit of targeted therapy eventually develop resistance to it and experience disease progression. This resistance, named extrinsic resistance (also known as secondary, evasive, acquired or adaptive resistance) has been explained more in detail [23]. TKI and VEGF inhibitors both target components of VEGF signaling pathway. Thus the mechanisms involved will affect any of these targeted agents. Extrinsic resistance results from the acquisition of adaptive mechanisms to the action of angiogenesis inhibitors which ultimately results in evasion of the angiogenesis and reemergence of tumor-related vasculature [3,25].

Sprouting of new vessels has been detected in Xenograft RCC tumors resected shortly after the start of sunitinib. The development of resistance is constantly preceded by restoration of blood flow, which suggests that new vasculature is less dependent (but not necessary independent) of VEGF [25].

4.2.1. Up-regulation of pro-angiogenic factors

Different pro-angiogenic factors involved in the mechanism of resistance to targeted agents have been recognized. In a mouse model of pancreatic neuroendocrine cancer, resistant tumors expressed high levels of FGF 1, 2, ephrin A1, angiopoetin and interleukin-8 [23,25]. Inhibition of these proteins was shown to inhibit tumor growth of resistant RCC-s [25].

Interleukin-8 (IL-8) is a potent pro-angiogenic factor. Up-regulation of IL-8 plays an important role in RCC resistance. In a xenograft model of RCC mimicking clinical resistance to sunitinib, increased IL-8 secretion from tumors was associated with reactivation of tumor angiogenesis and administration of IL-8 neutralizing antibody lead to re-sensitization to sunitinib. Elevated IL-8 expression was also found in patients with tumors who did not respond to sunitinib from the beginning [5,22]. IL-angiogenic signaling may functionally compensate for the inhibition of VEGF/VEGFR-mediated angiogenesis [5].

Angiopoetin 2 (Ang-2) is a plasma glycoprotein involved in angiogenesis and cancer neovascularization. It is thought to have a role in development of the resistance. Levels of Ang-2 decrease after the initiation of sunitinib treatment and increase after the resistance occurs [23].

Sphingosine kinase (S1P) is also supposed to play a role in the resistance. S1P is an enzyme that catalyzes the formation of sphingosine-1-phosphate which is associated with cell proliferation, survival and angiogenesis. Plasma levels of S1P decrease after the start of sunitinib treatment and increase again upon the development of resistance. In pre-clinical models administering neutralizing antibodies against S1P to mice, delayed the growth of sunitinib-resistant tumors [23].

4.2.2. Down-regulation of angiostatic factors

Down-regulation of angiostatic factors is another mechanism of resistance to TKI-s. Treatment with sunitinib and sorafenib results in the increased expression of several IFN-inducible genes including the angiostatic chemokines CXCL 10 and CXCL 11 and tumor suppressor genes. Following the development of resistance, the expression of IFN-γ and several of IFN-inducible genes is reduced. Down regulation of these factors is associated with the development of resistance to sunitinib and sorafenib [23].

4.2.3. Recruitment of bone marrow-derived cells

Recruitment of bone marrow-derived cells which can result in the development of new blood vessels is another possible mechanism of resistance. In pre-clinical studies recruitment of CD11b + GR1 + myeloid cells cells resulted in resistance development. There is also evidence that tumor vasculature can be protected from anti-angiogenic therapy by increased pericyte coverage [23].

4.2.4. Development of invasion without angiogenesis

Invasion of tumor in normal tissue and recruitment of normal tissue vasculature protect the tumors from anti-angiogenic therapy. It has been reported that the tumor of a patient experiencing disease progression during antiangiogenic therapy had invaded the surrounding tissue and there had been increase of the vascularization from the normal tissue to the center of the tumor [23].

4.2.5. Resistance to m-TOR inhibitors

Resistance to mTOR inhibitors is far less explained. It is supposed to be the result of activation of feedback loops that promote the activation of molecular signaling pathways of survival, increased activity of mTOR-complex 2, up-regulation of insulin-like growth factor and increase in the ERK/MAPK pathway signaling [4,24,25].

4.2.6. Reversible resistance

Preclinical studies revealed that resistance to VEGF targeted therapies can be reversible. Hammers and colleagues grafted skin metastases of mRCC patient who had become resistant to sunitinib into mice and these xenogafts regained sensitivity and responded to sunitinib. Histology of original skin metastasis and xenograft revealed that a reversible epithelial-to-mesenchymal transition could be responsible for acquired resistance to sunitinib. Zhang

concluded that reversible changes in gene expression within the tumor cells and/or their microenvironment could be the possible mechanism of reversible resistance. He implanted sorafenib-resistant RCC into mice and after implantation tumors regained the sensitivity to sorafenib [4,25].

4.2.7. Mechanism of resistance to different targeted agents

In the case of sunitinib, the proposed mechanism of resistance is the activation or up-regulation of alternative angiogenic signals (e.g. FGF-s, ephrins, andiopoetins) while in the case of sorafenib this mechanism seem to be recruitment of pro-angiogenic bone marrow-derived cells and monocytes. Recruitment of perycites that help to maintain vessels permeable and functional and prevent endothelial cells from being affected by antiangiogenic therapies, is the proposed mechanisms of resistance to pasopanib. In the case of bevacisumab resistance the increased potential of tumor cells to invade without the need of neovascularization is supposed to be the mechanism [4,9,25].

5. Overcoming the resistance

Overcoming the resistance to first line therapy is one of the aims of administering the second line and beyond. Several factors play important role in selection of second line strategy: clinical evidence, toxicity issues and individual patient profile [4,25,26].

Sequential use of targeted agents is currently the standard of care for mRCC patients. This approach enables patients to get most benefit from these agents avoiding the excessive toxicity associated with combination therapy [26-28]. Targeted agent in the second line can have the same or different mechanism of action as first-line one. Limited data suggest that the use of a TKI after the failure of another TKI is reasonable and that there is not complete cross-resistance of these agents. The hypothesis behind this is that although TKIs share the same mechanism of action, their molecular targets are different. Despite this, the evidence of this approach is not strong; prospective, phase III trials are missing. Changing mechanism of action can have several advantages: greater chance of overcoming resistance while decreasing the probability of cumulative toxicity [4,25]. Toxicities of TKI-s and mTOR inhibitors for example, differ considerably. Frequent grade 3 toxicities encountered in patients on TKI-s are hand-foot syndrome, diarrhoea, fatigue, hypertension, neutropenia and leukopenia, while grade 3 toxicities in patients treated with mTOR inhibitors are rash, stomatitis, pneumonitis, anemia and infection [25-30].

5.1. TKI-s following cytokine therapy

The almost historical treatment strategy where changing of mechanism of action proved to be effective was TKI-s following cytokines. Currently this approach is not of clinical use anymore, because most of the patients get molecular targeted agent in the first line; however it is likely that some patients will have been treated with a cytokine previously. Phase III trials demon-

strated that this approach is effective and safe and become a basis of approval of sunitinib and pazopanib in the first line treatment [3].

5.2. Combinations of targeted agents

Combinations of targeted agents could be in theory effective mechanism to overcome the resistance because we could combine agents with different mechanisms of action. However combining these therapies may increase the incidence of side effects if the combination drugs are not selected carefully [29]. Most of patients do not tolerate full doses of two VEGF inhibitors at the same time. That is the reason why administering combination therapy long enough to surpass the clinical benefit of subsequent mono-therapy is not possible [25].

Combinations of VEGF-TKI and mTOR inhibitors also lead to unacceptable toxicity. In a trial of Patel et.al, combination of temsirolimus and sunitinib lead to dose limiting toxicity in 2 of 3 patients [31]. Data suggest that the side effects and tolerability of combinations correlate with the total number of inhibited targets. This is the explanation why some combinations with VEGF specific agent bevacisumab may be tolerated (e.g. bevacisumab plus everolimus). At present combination of targeted agents in the treatment of mRCC is not recommended in clinical practice mainly because of excessive toxicity [23,29].

5.3. Second VEGF-TKI after the first line VEGF-TKI

Retrospective and prospective phase II trials showed that treatment with second TKI could be beneficial in patients that progressed on first TKI. At first sight this may seem not logical, but variations in kinase targets and interaction may avoid resistance. However definitive data from phase III trials on this topic are still missing. Benefit of the second TKI after the first TKI may be dependent on its relative potency and selectivity profile [9]. Most of the results from retrospective and small prospective trials suggest that patients with mRCC who progress on sorafenib could benefit from sunitinib. Conversely the use of sorafenib after sunitinib or bevacisumab showed limited efficacy [9,27].

Sabin et.al. evaluated 68 patients treated with sunitinib and sorafenib consequently. ORR was better when the patients received sorafenib first; 15% in the group that received sunitinib followed by sorafnib group and 9% in the group that received sorafenib after sunitinib. Median PFS in the first group was 12.4 months (6 months on sorafenib and 6.4 months on sunitinib) and 8.9 months in the second group (5 months on sunitinib and 3.9 months on sorafenib) [26]. Porta et.al evaluated retrospectively 99 patients treated with sunitinib followed by sorafenib (SuSo) and 90 patients treated with sorafenib followed by sunitnib (SoSu). The median PFS of second line treatment in the first group (SuSo) was 7.9 months and in the second group was 4.2 months (SoSu) [32]. Clinical trial in progress NCT00732914 with the aim to evaluate if total PFS of sorafenib followed by sunitinib is superior compared to sunitinib followed by sorafenib is expected to give some additional light to this issue [3].

AXIS trial directly compared the efficacy and safety of axitinib to sorafenib after progression on sunitinib, bevacisumab, temsirolimus or cytokines. In the subpopulation of patients who previously received sunitinib, median PFS was 4.8 months with axitinib and 3.4 months with

sorafenib (p=0,001). Shorter median PFS in both arms receiving first line sunitinib compared to those receiving cytokines (median PFS 12.1 in axitinib and 6.5 moths in sunitinib) suggest that at least partial cross-resistance with sequential TKI-s [3,9,19].

Reduced clinical efficacy of second line therapy as a result of cross resistance is key concern associated with the sequential administration of agents targeting the same molecular pathways. Two prospective trials showed that because of the cross-resistance, sorafenib had limited efficacy in patients who progressed on sunitinib or bevacisumab [27].

Another concern about using sequential VEGF-TKI therapies is toxicity. Although they may differ in toxicity profiles, all TKI-s share similar targets and exhibit class effect toxicities like hypertension, hand foot syndrome and rash [9]. Current data suggest that Switching to agents with different mechanisms of action in the second line therapy may provide superior efficacy and reduced cumulative toxicity [27].

5.4. VEGF-TKI after first line anti VEGF

Very limited data are available on the use of TKI-s after progression on bevacisumab and no clinical trial is currently ongoing to address this issue. Only two minor prospective trials conducted by Garcia and Rini evaluated the use of sunitinib or sorafenib in patients with bevacisumab-refractory mRCC [3].

In a phase II trial of Garcia, 48 patients were enrolled. After progression on treatment with sunitinib or bevacisumab, patients received twice daily 400 mg of sorafenib. One unconfirmed objective partial response was observed and the tumor burden reduction rate was 30%. The median PFS was 4.4 months. There was no association of PFS and tumor shrinkage with response to prior therapy. Most treatment-related adverse events were of mild-to-moderate intensity, and included fatigue, hypertension, diarrhoea, and hand-foot syndrome [33].

Rini et.al. conducted a phase II multicentric trial in which patients with mRCC and disease progression after bevacizumab-based therapy received oral sunitinib 50 mg once daily in 6-week cycles on a 4/2 schedule (4 weeks with treatment followed by 2 weeks without treatment). Sixty-one patients were enrolled. The ORR was 23.0%, median PFS was 30.4 weeks and median OS was 47.1 weeks. Most treatment-related adverse events were of mild-to-moderate intensity and included fatigue, hypertension, and hand-foot syndrome. Results from measuring different VEGF-s in the plasma suggest that sunitinib could inhibit some of the signaling factors involved in bevacisumab resistance [34].

5.5. mTOR inhibitor after first line VEGF-TKI

Another approach in patients who progress on first line TKI-s is to switch to a second line therapy with an agent with different mechanism of action like mTOR inhibitor [3,9]. On theoretical basis mTOR inhibitors could overcome the resistance to VEGF-TKIs. VEGF-TKIs increase tumor hypoxia which results in up-regulation of proangiogenic factors and increase potential of metastases. mTor inhibition decreases translation of proangiogenic factors and tumors that have become resistant to VEGF-TKI may respond to treatment with mTOR

inhibitor [27]. The evidence of effectiveness of this approach comes from preclinical data. Trial conducted by Larkin and colleagues compared treatment with sunitinib, sunitinib followed by sorafenib or sunitinib followed by everolimus in mice implanted with murine RCC. Sunitinib followed by everolimus was associated with reduced primary tumor weight and volume in a greater extend compared to tumors treated with sunitinib and sunitinib followed by sorafenib. The conclusion was that sequential therapy with sunitinib followed by everolimus is associated with significant anti-tumor and anti-metastatic effect [35].

Everolimus was approved in the second line therapy on results of RECORD-1 trial. In this double blind, phase III trial, patients who had progressed on first line sunitinib, sorafenib or both were randomized to everolimus or placebo. Patients receiving everolimus had longer PFS compared to placebo (4.9 vs. 1.9 months, p<0.001). The clinical benefit of everolimus was observed regardless if the patients received previously one or two consequent TKI-s. In the subgroup of patients who received one TKI, median PFS in everolimus group was 5.4 months, and in group who received two TKI-s 4 months. This was statistically significant longer than in placebo groups, where PFS was 1.9 and 1.8 months respectively [8,20,36-38].

Prospective head to head trials to compare mTOR inhibitors and VEGF-TKI-s in the second line of treatment in patients who progressed on the first line VEGF-TKI-s have not been done. Di Lorenzo and colleagues indirectly compared survival benefit in patients on everolimus or sorafenib in the second line. Median overall survival was 81.5 weeks for patients receiving everolimus and 32.0 weeks for sorafenib [37].

The optimal sequencing of sunitinib and everolimus is currently being evaluated in the RECORD-3 trial and furthermore the everolimus plus bevacisumab in the second line after progression on TKI-s is currently being compared to everolimus plus placebo in the NCT01198158 trial [3].

The efficacy and safety of temsirolimus after progression on TKI-s are expected to be revealed in an ongoing trial NCT00474786, a phase III trial comparing temsirolimus vs. sorafenib in the second line treatment in patients who have failed on first-line sunitinib. Results from small population in retrospective and prospective phase II trial presented on ASCO 2010 suggest, that temsirolimus is safe and effective in pretreated patients, especially those with good performance status and good prognostic factors [3].

Regarding toxicity mTOR inhibitors and VEGF-TKIs block different molecular mechanisms, the toxicity profiles are usually not overlapping. In the RECORD-1 trial patients could tolerate treatment with everolimus after progression on VEGF-TKIs. Stomatitis, infection, asthenia and fatigue were the most common side effects reported on everolimus therapy. Common toxicities encountered in the treatment with VEGF-TKIs such as hypertension or hand-foot syndrome, were not frequent [3,19].

5.6. Alternative scheduling and dosage

A different approach to overcome the resistance can potentially be the change in scheduling and/or dosage of the targeted agent in usage. Sunitinib is approved in intermittent schedule of 4 weeks on drug and 2 weeks off drug. Continuous low-dose therapy has been shown to be

a feasible treatment option in first and second line of treatment [37,38]. Comparison between the two scheduling is currently not very well determined, but clinical and toxicological differences may in future be important issue in treatment individualization. Another option is re-challenge with the same drug after discontinuation period on disease progression. The basis for this approach comes from pre-clinical data that indicate that resistance to sorafenib is reversed by re-implantation of resistant tumors in untreated mice [23].

The question of optimal treatment dosage becomes particularly relevant on disease progression. Meta-analysis of patients with solid tumors receiving sunitinib revealed that patients receiving higher dose, had longer time to progression compared to patients who received less sunitinib. Additionally patient receiving higher dose had more complete or partial remissions and greater decrease in tumor size. In the trial comparing sorafenib with IFN-α, patients in the sorafenib group received higher dose of sorafenib (600 mg BID) after progression on 400 mg BID. Reduction of tumor size was observed. Suggested clinical benefit of increased dose after progression is outweighed by increased toxicity. Most of patients do not tolerate dosage increase [22].

5.7. Intrinsic resistance

Prognosis of patients who progress early in the course of first line therapy VEGF targeted therapy is poor. No available agents seem to alter the course of their disease and give them clinical benefit. 86 patients with rapid progression after first line therapy were evaluated in a retrospective trial. PFS after second line therapy with treatment with different VEGF-TKI was 2 months and after second line therapy with mTOR 0.9 months (p=0.536). Larger retrospective trial in which 272 patients were included showed similar results. All patients had rapid disease progression after first line VEGF-TKIs. The response rates, PFS and OS of those receiving second-line VEGF-targeted therapy compared with mTOR inhibitors were 10 vs 6%, 2.8 vs.2 months and 7.9 vs. 4.7 months. Differences were not statistically significant [9].

6. Third line and beyond

Small prospective and retrospective trials suggest that changing the mechanism of action in the third line may restore the sensitivity to the initial treatment [3,9,27,32,39,40]. In the ASCO meeting in 2010 Ferrari presented the results of a prospective trial that compared the administration of everolimus or temsirolimus as third line therapy to good performance status patients resistant to TKI-s. Median PFS was 6 months and disease control was achieved in 39% of patients. These results suggest that treatment with mTOR inhibitor in the third line and further than, could be a potential promising treatment option [40].

Another trial conducted by Di Lorenzo et.al. evaluated sorafenib treatment in the third line after treatment with sunitinib and mTor inhibitor. Of the 34 patients eligible, 23.5% responded to third line sorafenib. Desease control was 44%, median PFS was 4 and median OS was 6 months. 47% of patients that responded to first line therapy, responded to third-line sorafenib while of patients who did not respond to first-line, did not respond also to third-line sorafenib.

The most common 3/4 grade side effects of third line sorafenib were hand-foot syndrome, anemia, fatigue, diarrhea and neutropenia. These results show that sorafenib could be considered in the third line treatment in mRCC patients after the failure on sunitinib and mTOR inhibitor [41]. Blesious retrospectively evaluated 105 patients in the RECORD-1 trial of whom 36 received a VEGF-TKI after receiving everolimus. Patients that received sunitinib, sorafenib and dovitinib had median PFS of 8 months, 5.3 months and 12.0 months. A partial response was reported in 8.6% of patients and 68.8% of patients had stable disease. Median OS was 29.1 months [42].

In a trial of Grunwald efficacy of VEGF-targeted therapies in patients after everolimus-resistant patients who had progressed on a previous TKI was explored. Patients received sunitinib, sorafenib, dovitinib or bevacisumab/ IFN therapy after failure of everolimus. Of the 40 patients included 10% had partial response and 55% had stable disease. Median PFS was 5.5 months. Authors conclude that VEGF targeted therapy show promising activity in everolimus resistant metastatic RCC [43].

Porta et. al. evaluated retrospectively the overall PFS benefit of the sequence VEGF-TKI, mTor inhibitor, VEGF-TKI sequence. The sequence of sorafenib-mTOR-sunitinib (14 patients) was compared to the sequence sunitinib-mTOR-sorafenib (26 patients). No significant difference in PFS was found between the two groups (21.9 months vs 22.8 months). The median PFS for the three lines of treatment were 11.7 months-5.1 months-9.1 months for the group sorafenib-mTOR-sunitinib and 14.4 months-4.3 months-3.9 months for sunitinib-mTOR-sorafenib group [44].

The results of these trials suggest that re-challenging strategy of VEGF-TKI in the third line of treatment after progression on VEGF-TKI in the first and mTOR inhibitors in the second may be a successful treatment approach. The other observation of these trials is that some patients have minor benefit from the VEGF-TKI inhibitors in the third compared to the benefit in the first line. The explanation for this is that probably partial cross-resistance to the VEGF-TKIs accounts at least to some extent for this. One possible strategy to overcome this resistance is to use the third-line agent with the ability to broadly inhibit multiple angiogenic pathways in addition to VEGF signaling [9].

Even if no clinical guidelines exist for the fourth-line targeted therapy, some reports suggest that patients may gain clinical benefit from the sequences of targeted agents with different mechanisms of action. 48 months of PFS was achieved in an mRCC patient treated with four lines of targeted therapies (sunitinib, everolimus, sorafenib, temsirolimus). Despite intensity, treatment was well tolerated and no cumulative toxicity was present. This case study advocates that sequential use of sunitinib, everolimus, sorafenib and temsirolimus and show that this could be effective treatment approach with good toxicity profile [45].

Metastatic RCC patients can get benefit from multiple lines of targeted therapy. Resistance to VEGF-TKIs and mTOR inhibitors seem to be at least partially reversible and re-challenging with the inhibitors from the same group in subsequent lines of therapy may be a therapeutic option if toxicity does not limit it. Tailoring treatment to the particular patient is of utmost importance [3,9,27,32].

7. Future directions

The current practice of delivery of sequential monotherapy targeted agents is empirical and mainly based on non-comparative clinical trials. In treatment refractory patients often practical issues like route of delivery or physician familiarity with the drug prevail over the scientific evidence in selecting treatment. Deeper understanding of the biology of response and resistance to targeted agents will elucidate future way in treatment of mRCC patients. New multi-targeted inhibitors are being rapidly developed and their role in overcoming resistance will become clear in the next few years. Together with the developments of these new drugs, finding predictive biomarkers of these new and "old" therapies is one of the major research goals [25].

7.1. New targeted agents

Dovitinib is an investigational multi-target inhibitor of FGF receptors 1-3, PDGF receptor, VEGFrs 1-3 and c-KIT. One of the mechanisms of resistance to VEGF-TKI seems to be hypoxia-mediated induction of FGF signaling. Dovitinib was tested in a phase II trial in which patients with mRCC who failed prior treatment with VEGF-TKI or mTOR inhibitor or cytokines. Median PFS and OS were 5.5 months and 11.8 months in all patients and 6.1 months and 10.2 months in patients treated with previous VEGF-TKI or mTOR inhibitor. The main grade 3 toxicities of dovitinib were nausea/vomiting in 15%, fatigue in 13.6%, asthenia in 13.5%, diarrhoea in 10.2% and hypertension in 10.2% of patients. Grade 4 hypertriglicemia occurred in 8.5% of patients [46].

Tivozanib is a potent selective long half-life tyrosine kinase inhibitor targeting VEGFR 1-3. 517 patients were included in a phase III trial published by Motzer et.al at ASCO 2012. Patients were randomized to receive either tivozanib or sorafenib. Median PFS was 11.9 months for tivozanib and 9.1 months for sorafenib (p=0.042). Overall response rate was 33% in tivozanib and 23% in sorafwnib group (p=0.014). Adverse events grade 3 for tivozanib were hypertension, diarrhea, fatigue, neutropenia and hand-foot syndrome [47].

7.2. Biomarkers

Predictive biomarkers could help clinicians to determine the best treatment approach [48,49]. Multiple candidate biomarkers of biological tumor activity as well as treatment response and patient prognosis are being evaluated [49]. However up to date, none of them showed potential in clinical use. VHL gene status did not correlate with PFS or OS. The reason for this may be that almost all RCC cancers have VHL silencing and so this marker cannot be selective enough. Biomarkers in the peripheral blood have also been tested. Results of some trials showed that patients with mRCC and elevated expression of angiogenic factors have greater benefit from VEGF-targeted therapies, although other trials yielded inconsistent results. Other types of predictive markers, like changes in the tumor blood flow measured by MRI during treatment with targeted agents are being explored [25,48,49].

8. Conclusion

Despite great improvement in treatment outcomes with targeted agents in mRCC, the fact remains that complete remissions are rarely achieved and most patients progress and develop resistance to the treatment. Many questions are still open and at least some of them are expected to be solved with the on-going and future clinical trials. Intrinsic and extrinsic tumor resistances are major obstacles in successful long term tumor control and one of the major questions is the optimal sequencing of treatment. Use of sequential therapy with changing mechanisms of action is a rational approach to overcome this resistance.

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